

53. (new) The method of claim 52 wherein the concentration of the boric acid derivative is about 50 to 200 mM.

### REMARKS

In view of the preceding amendments and the comments which follow, and pursuant to 37 CFR §1.111, amendment and reconsideration of the Official Action of December 14, 2001 is respectfully requested by Applicant.

#### Amendments to claims

Claim 28 has been amended to clarify antecedent basis for "analyte" in claim 26. New claims 46-53 drawn to a method for stabilizing a solution comprising a hydrogen-accepting coenzyme have been added. Support for the new claims is found throughout the specification and in claims 18-25.

"Clean" and "marked up" versions of currently pending claims 18-53 are attached hereto for the Examiner's convenience.

#### Rejection under 35 USC §112

The Examiner has rejected claim 28 under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The Examiner points out that claim 28 recites the limitation "the analyte" in line 1 of the claim and that there is insufficient antecedent basis for this limitation in the claim.

In response, Applicants have corrected this reference by replacing "analyte" with "hydrogen-transferring substrate". The rejection is now avoided, and the Examiner's reconsideration is respectfully requested.

Rejection under 35 USC §103 (a)

The Examiner has rejected claims 18-45 under 35 USC §103 (a) as being unpatentable over U.S. Patent No. 5,424,204 issued to Aoyama *et al.* (hereinafter "Aoyama"). The Examiner argues that Aoyama discloses an assay reagent for determining the concentration of glucose, the concentration of a coenzyme or for measuring the activity of glucose-related enzyme. The reagent comprises a glucose-6-phosphate dehydrogenase and a stabilizer such as a hydroxylamine derivative. Aoyama discloses that the hydroxylamine is included in the solution in an amount between 0.001 to 1 mol/l. Aoyama discloses that the reagent may additionally comprise a coenzyme such as NAD and NADP and a buffer including citrate and borate buffers. Aoyama teaches that a buffer may be included at a concentration between 0.005 to 2 mol/l and such that the pH is between 3 and 11. Aoyama further teaches that the amount of NADPH formed is measured by determining the change in absorbance at 340 nm.

Aoyama does not provide a specific example of a solution containing glucose-6-phosphate dehydrogenase and a hydroxylamine derivative in addition to a citrate or borate buffer. However, the Examiner argues, Aoyama does teach an aqueous reagent comprising glucose-6-phosphate dehydrogenase and a hydroxylamine derivative and teaches that the dehydrogenase and the hydroxylamine may be included in buffer solutions selected from citrate and borate buffers. It is the Examiner's position that it would therefore have been obvious to one having ordinary skill in the art at the time of the invention to have prepared an aqueous reagent including a dehydrogenase enzyme, a coenzyme and hydroxylamine as a stabilizer in buffer solution and to have utilized the solution for determining glucose concentration, for determining coenzyme concentration or for measuring the catalytic activity of an enzyme according to the specific teachings of Aoyama. It would additionally have been obvious to one having ordinary skill in the art to have prepared kits including an enzyme, a coenzyme, a hydroxylamine and an appropriate buffer as specifically taught by Aoyama.

In rebuttal, Applicants point out that the object of Aoyama is to stabilize the enzyme glucose-6-phosphate dehydrogenase in a liquid clinical assay reagent which is used for quantitatively determining the amount of a glucose-related substance (column 1, lines 51-56). The enzyme lacks stability for practical applications (column 1, lines 29-33). To stabilize the fragile enzyme, Aoyama's reagent comprises the enzyme glucose-6-phosphate dehydrogenase and a stabilizer such as a hydroxylamine derivative (column 9, claim 1). Again, it is of importance that Aoyama seeks to stabilize the enzyme itself, which is part of the reagent. Aoyama mentions the addition of common buffers like citrate or borate buffer to his glucose-6-phosphate dehydrogenase-containing reagent (column 6, lines 27-34). He also mentions the possible addition of known coenzymes like NAD or NADP (column 6, lines 12-14). But Aoyama does not disclose a reagent containing a coenzyme and organic compounds having a pKa value between 1.5 and 6.0 and a nitrogen compound of the formula recited in Applicant's claim 18 (hydroxylamine derivative). Aoyama just mentions typical additives to a reagent solution but there is no hint to provide a reagent containing a stabilized coenzyme itself. Aoyama provides no motivation to try an organic compounds having a pKa value between 1.5 and 6.0 and a hydroxylamine derivative of the formula recited in Applicant's claim 18 because Aoyama is concerned with stabilization of an enzyme. Aoyama is completely silent with regard to stability problems of a coenzyme.

In contrast, the object of Applicant's invention is to stabilize the coenzyme, e.g., NAD, NADP and derivatives, even if no enzyme is present in the reagent solution. Applicant's invention provides a stable blank value even if the reagent is stored under stress, i.e., high temperature, conditions before using it.

As explained above, Applicants argue that the Examiner has failed to make a *prima facie* case of obviousness, and reconsideration of the rejection of claims 18-45 is respectfully requested.

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With respect to Aoyama and newly added claims 46-53, Aoyama does not disclose or suggest, nor does Aoyama provide motivation to try, a method for stabilizing an aqueous solution comprising a hydrogen-accepting coenzyme as recited in claim 46 and claims 47-53 depending therefrom. Thus, the Examiner's case of *prima facie* obviousness argued against claims 18-45 likewise cannot be made against new claims 46-53.

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Applicants submit that their application is now in condition for allowance, and favorable reconsideration of their application in light of the above amendments and remarks is respectfully requested. Allowance of claims 18-53 at an early date is earnestly solicited.

The Examiner is hereby authorized to charge any fees associated with this Amendment to Deposit Account No. 02-2958. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

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